# Direct Detection of a Sulfonate Ester Genotoxic Impurity by Atmospheric-Pressure Thermal Desorption−Extractive Electrospray−Mass Spectrometry

Neil A. Devenport,[†] Laura C. Sealey,[†] Faisal H. Alruways,[†] Daniel J. Weston,[‡] James C. Reynolds,*[,†] and Colin S. Creaser*[,†]

[†]Centre for Analytical Science, Department of Chemistry, Loughborough University, Leicestershire, LE11 3TU, United Kingdom
[‡]DMPK Innovative Medicines, Oncology iMed, AstraZeneca R&D, Alderley Park, Cheshire, SK10 4TG, United Kingdom

**ABSTRACT:** A direct, ambient ionization method has been developed using atmospheric pressure thermal desorption−extractive electrospray−mass spectrometry (AP/TD-EESI-MS) for the detection of the genotoxic impurity (GTI) methyl p-toluenesulfonate (MTS) in a surrogate pharmaceutical matrix. A custom-made thermal desorption probe was used to the desorb and vaporize MTS from the solid state, by rapid heating to 200 °C then cooling to ambient temperature, with a cycle time of 6 min. The detection of MTS using EESI with a sodium acetate doped solvent to generate the $[MTS+Na]^+$ adduct ion provided a significant sensitivity enhancement relative to the $[M+H]^+$ ion generated using a 0.1% formic acid solvent modifier. The MTS detection limit is over an order of magnitude below the long-term daily threshold of toxicological concern (TTC) of 1.5 $\mu$g/g and the potential for quantitative analysis has been determined using starch as a surrogate active pharmaceutical ingredient (API).



(cross section of AP-TD/EESI set-up)
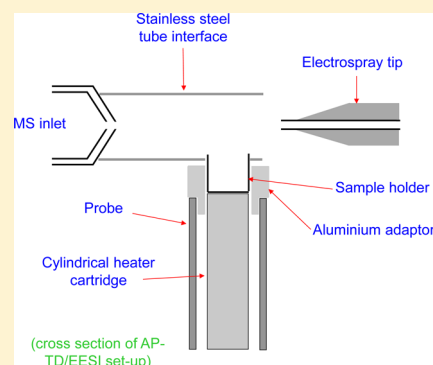
The ability to detect genotoxic impurities (GTIs) at low concentrations in active pharmaceutical ingredients (APIs) is important in the development of APIs.[1] The use of sulfonic acids as counterions during salt crystallization can result in undesired reactions with alcohols to form sulfonate esters.[2] The genotoxicity of sulfonic acid esters was reported by Glowienke et al. in 2005, based on in vitro salmonella reverse mutation (Ames)[3] and micronucleus tests.[4] The Ames test indicated that most sulfonic acid esters generated at least a 2-fold increase of revertants, i.e., his⁻ auxotrophs to his⁺ prototrophs, in relation to controls. The micronucleus assay supported these findings with most compounds showing genotoxic properties due to the numbers of micronuclei (chromosomal aberrations) formed within the cultured mouse lymphoma cells (L5178Y), in comparison to controls.[5] The United States Food and Drug Administration (USFDA) and Europe's European Medicines Agency (EMA) have established a threshold of toxicological concern (TTC) of 1.5 $\mu$g day⁻¹ (1.5 ppm, assuming a daily dose of 1 g) for long-term treatments and a staged TTC where increased levels for limited periods are allowed for the purpose of drug development.[6] The international conference on harmonization, Q3A(RS) and M7 (step 2),[7,8] has outlined the industry requirements for the qualification of GTIs in drug products. The pharmaceutical industry must demonstrate control and evaluation of raw materials (to a given threshold) that have the potential to form genotoxic impurities during drug production.[9−11] The case for control of sulfonate salts can be exemplified by Nefinavir mesilate, which is sold under the brand name of Viracept, produced by Roche, where an error in production generated elevated levels of sulfonate esters in the final product, which was subsequently withdrawn from the European markets.[12]

The threshold limit applied to sulfonate esters has led to the development of several analytical approaches for the determination of these GTIs.[13] The most widely used method is gas chromatography (GC) combined with mass spectrometry (MS)[14,15] or flame ionization detection (FID).[16] The derivatization of sulfonate esters prior to headspace GC-MS,[17,18] and sample preconcentration using solid phase microextraction (SPME)[19] have also been employed to achieve the levels of sensitivity required. Other approaches include liquid chromatography−mass spectrometry (LC-MS) and liquid chromatography−tandem mass spectrometry (LC-MS/MS).[20−22] The ability to accurately detect the sulfonate esters is limited by their relative stability, since the compounds may degrade during sample preparation, leading to poor analytical sensitivity; therefore, rapid detection of these compounds is advantageous. To date, the application of direct analysis techniques to the determination of GTIs in pharmaceutical formulations has received little attention. The mesylate, besylate, and tosylate sulfonates are low-molecular-weight compounds with boiling points that are generally below 200 °C, indicating that thermal desorption (TD) could be employed as a possible sampling mechanism. The potential of GTI detection by direct analysis was recently demonstrated

in 2011 by McCullough et al., using online extractive electrospray (EESI) sampling from a heated reaction mixture, where codeine was used as a surrogate GTI.[23]

In this paper, an in-house designed and constructed TD probe capable of heating solid and liquid samples to temperatures in excess of 350 °C was interfaced to a quadrupole-time-of-flight-mass (Q-ToF) spectrometer. The desorbed methyl *p*-toluenesulfate ester (MTS) vapor was detected as a sodium salt adduct ionized by extractive electrospray[24] in the presence of 0.01 M sodium acetate. This novel approach to the direct detection of MTS in a surrogate API mixture (starch) is demonstrated to levels below a TTC of 1.5 $\mu$g/g required by the FDA and EMA regulatory bodies.

## ■ EXPERIMENTAL SECTION

**Chemicals.** Analytical-grade water and methanol were purchased from Fisher Scientific (Loughborough, U.K.). HPLC grade formic acid, sodium acetate ($\geq$99.0%), methyl *p*-toluenesulfonate ($\geq$97.0%) and potato starch were purchased from Sigma−Aldrich (Gillingham, U.K.). All chemicals were used without further purification.

**AP/TD Probe.** A schematic diagram of the ion source region of a Waters Synapt HDMS spectrometer (Waters, Manchester, U.K.) modified for AP/TD-EESI-MS is shown in Figure 1. The
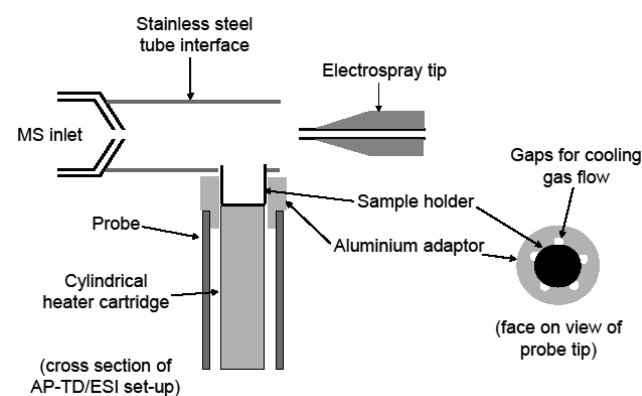


**Figure 1.** Schematic diagram of the AP/TD-EESI-MS ion source region (not to scale).

TD probe was constructed by placing a cylindrical heater cartridge (2.5 mm o.d. × 50 mm; RS Components Ltd., Northants, U.K.) into a stainless steel tube (12 mm i.d. × 300 mm) with the heater positioned inside an aluminum adaptor at the probe tip. Samples were placed in a brass sample holder (o.d. 2.5 mm × 10 mm) located within the aluminum adaptor, with the base of the sample holder in direct contact with the heater cartridge. The sample holder was inserted through a hole in the side of a stainless steel tube (o.d. 12 mm × 50 mm) located between the ESI probe and the mass spectrometer inlet cone that spatially confines the thermally desorbed analytes for ionization by an in-line electrospray plume. The cartridge temperature was set using a regulated temperature controller (Tempatron, Reading, U.K.) capable of a maximum operating temperature of 400 °C. The operating temperatures set to 250 °C resulted in experimental temperatures of >190 °C within 2 min. The AP-TD probe was cooled using a flow of chilled air (10 L min$^{-1}$ at 10 °C) through the probe.

**Sample Preparation.** Methyl *p*-toluenesulfonate standards were prepared in methanol at concentrations of 0.5−10 ng $\mu$L$^{-1}$. [*Note: MTS is genotoxic and should therefore be handled in*

*low volumes within a fume hood, using suitable personal protection.*] This was performed by incineration. In the TD experiments, MTS standards were placed into the sample holder and immediately introduced into the ion source. For surrogate API experiments, 10 $\mu$L of standard solution was added to 50 mg of starch, equivalent to concentrations in the range 0.1−2 ppm (*w/w*), using aluminum foil sample wraps which were prepared prior to analysis and placed into the sample holder. A small hole was made in the aluminum foil immediately before thermal desorption.

**EESI-MS Conditions.** The EESI solvent consisted of 50:50 MeOH:H$_2$O (*v/v*) modified with 0.1% formic acid (*v/v*) in initial studies and 0.01 M sodium acetate (NaOAc) in all other investigations, which were infused from a syringe pump at a flow rate of 8 $\mu$L min$^{-1}$. The mass spectrometer (Synapt HDMS, Waters, Manchester, U.K.) was operated in sensitivity (V) mode, using positive ionization with a capillary voltage of 3 kV and a cone voltage of 20 V. The source and desolvation temperatures were set to 150 and 120 °C, respectively, and the source gas flow rate (N$_2$) was set to 20 L h$^{-1}$.

## ■ RESULTS AND DISCUSSION

The application of atmospheric pressure thermal desorption−extractive electrospray−mass spectrometry (AP/TD-EESI-MS) to the analysis of a GTI with the thermal desorption probe introduced into the modified ion source region of the Q-ToF spectrometer was evaluated using methyl *p*-toluenesulfonate (MTS). The responses of the protonated and sodiated MTS ions generated by extractive electrospray[24] were monitored during the introduction of thermally desorbed MTS vapor into the ion source. The mass spectra of MTS acquired using a methanol/water EESI plume with the addition of 0.1% formic acid (*v/v*) and 0.01 M NaOAc as solvent-modifying agents are shown in Figure 2.

Figure 2a shows the MTS response using 0.1% formic acid as the solvent modifier with the [M+H]$^+$ ion at *m/z* 187.0440 (5.8 ppm mass error) present as the base peak. However, the protonated MTS fragments readily in the interface of the mass spectrometer yielding products at *m/z* 155, assigned to [MH−CH$_3$OH]$^+$, an unidentified rearrangement ion at *m/z* 127 and the tropylium ion at *m/z* 91, which reduces the sensitivity for the [M+H]$^+$ ion. The presence of an [M+Na]$^+$ ion in the mass spectrum (Figure 2a) indicates an ionization process involving conversion of a gas-phase (neutral) MTS molecule into a sodiated gas phase ion by interaction with a solvent droplet containing traces of sodium in the EESI plume. The co-ordination of Na$^+$ with tosylate molecules has been reported by Bai et al., who demonstrated that detection was improved by the formation of alkali metal adducts.[21] Doping the EESI solvent with 0.01 M sodium acetate exclusively yielded the [M+Na]$^+$ ion for MTS (*m/z* 209.0258; 4.8 ppm mass error; Figure 2b). The absence of the [M+H]$^+$and its associated fragments in the mass spectrum improves analyte sensitivity increasing the mass spectral response by a factor of ~2 for the [M+Na]$^+$ ion using 0.01 M sodium acetate compared to the formic acid doped EESI plume and no fragmentation of the sodiated ion was observed. A sodium-doped EESI plume was used in all subsequent experiments.

The TD probe can achieve a temperature of ~200 °C in 2 min, which was found to be sufficient for the desorption of MTS vapor. After reaching the maximum desorption temperature, the probe was cooled by a flow of chilled air. The flow of cooled gas passed through the probe and exited at the probe
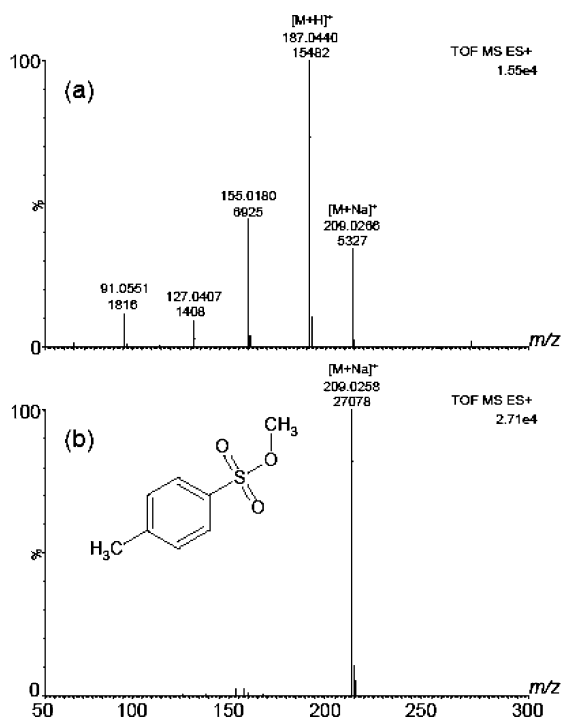
**Figure 2.** Mass spectra obtained using thermal desorption of MTS vapor into the modified EESI ion source (50:50 ($v/v$) MeOH:$H_2O$) with (a) 0.1% formic acid ($v/v$) solvent modifier and (b) 0.01 M sodium acetate modifier.

tip, which rapidly cooled the sample holder to ambient temperatures with a total run time of 5 min.

The AP/TD-EESI-MS analysis of MTS was carried out with the GTI spiked into 50 mg of starch to simulate the environment of an API. The samples were preprepared using sealed aluminum foil wraps that were pierced prior to analysis. The use of the disposable aluminum wraps prevented sample cross contamination and provided a rapid method of exchanging samples, reducing sample to sample cycle time to 6 min, which is significantly shorter than previously reported GC-MS and LC-MS run-times of 24 and 11 min, respectively.[12,17] An example of the thermal desorption profile and mass spectrometric response obtained for the AP/TD-EESI-MS analysis of MTS in starch is shown in Figure 3.

The total ion response for a 50 mg starch sample (Figure 3a), used as a surrogate API, spiked with MTS at a level of 2 ppm initially increases with the probe temperature and then decreases when the heat is removed and the cooling gas flow initiated. The ion response returns to baseline levels within 4 min, but the temperature of the sample holder at this point is still too high to be handled ($\sim$70 °C) and requires an additional 1 min of cooling. The selected ion response for the sodiated MTS ion ([M+Na]$^+$, 209.02 $\pm$ 0.02) is shown in Figure 3b. The volatility of the MTS provides a sharp desorption peak, with a peak width at half height of 15 s. The maximum response for MTS is observed at 0.9 min when the TD probe temperature was $\sim$100 °C. The MTS response returns to baseline levels within 3 min. The mass spectrum obtained from the MTS desorption peak is shown in Figure 3c. The background-subtracted spectrum, averaged across the peak at half height, shows a base-peak response for the sodiated MTS ion (7.7 ppm mass error). The application of AP/TD-EESI-MS removes the requirement for lengthy sample
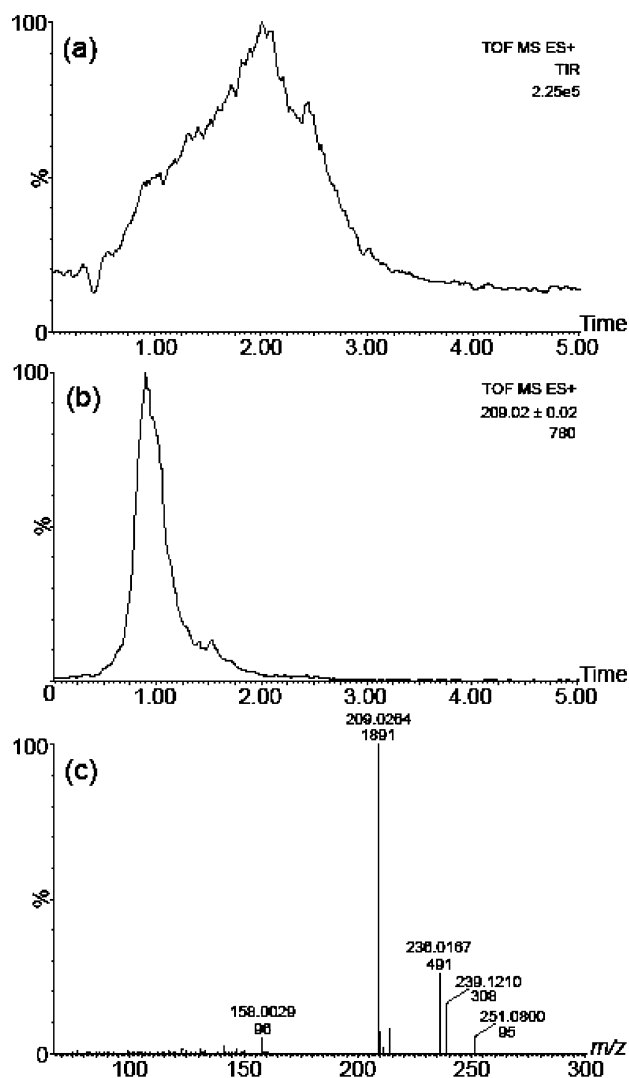


**Figure 3.** AP/TD-EESI-MS analysis of MTS (2 ppm (w/w); 100 ng MTS in 50 mg starch) (a) total ion response; (b) selected-ion response for the sodiated MTS adduct ion ($m/z$ 209.02 $\pm$ 0.02); and (c) summed, background subtracted mass spectrum for MTS.

preparation and derivatization steps associated with other MTS detection techniques;[15,16] because low-volatility APIs will not be desorbed by TD, and sample throughput is maximized by reducing total analysis time and using disposable sample holders.

The AP/TD-EESI-MS technique has a limit of detection (S/N 3:1) at 0.1 ppm (0.1 $\mu$g/g), which is 15 times lower than the limit set by the FDA and the EMA allows the detection of MTS (3:1, signal:noise). The correlation coefficient ($R^2$ = 0.983) and the percentage relative standard deviation (%RSD) of 22% for the MTS peak areas at the 0.1 ppm level, are affected by the requirement to remove and replace the TD probe in the AP/TD-EESI source for each individual sample. The %RSD and linearity values are acceptable for such a manual analytical technique, used without an internal standard, but they do not yet meet the requirements for the use of the AP/TD-ESI-MS technique for quantitative measurements.[25] The direct analysis method exceeds the detection requirements for MTS, indicating its potential as a rapid screening procedure based on a limit test to show that the MTS is below the TTC level. This application would be a useful indicator of the presence/

absence of potential impurity, which could then be determined quantitatively using a well-established technique such as GC-MS.

## CONCLUSIONS

The direct detection of MTS in a surrogate API matrix is demonstrated using a TD probe combined with electrospray-mass spectrometry. This direct, ambient ionization approach offers reduced sample preparation and analysis times compared to previous GC and LC techniques, enabling high throughput analyses. The thermal desorption of MTS from a surrogate API matrix and co-ordination with sodium to form a stable vapor-phase sodium adduct ion provides levels of sensitivity that are greater than the regulatory requirements for this GTI. The technique has potential application to the screening of APIs for MTS, and potentially other alkyl sulfonate esters, formed during the pharmaceutical manufacturing processes.

## AUTHOR INFORMATION

**Corresponding Author**

*Tel.: +44 (0)1509 222552. Fax: +44 (0)1509 223925. E-mail. C.S.Creaser@lboro.ac.uk (C.S.C.), J.C.Reynolds@lboro.ac.uk (J.C.R.).

**Notes**

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

## REFERENCES

(1) Teasdale, A. *Genotoxic Impurities: Strategies for Identification and Control*; Wiley: Hoboken, NJ, 2010.

(2) Elder, D. P.; Teasdale, A.; Lipczynski, A. M. *J. Pharmaceut. Biomed. Anal.* **2008**, *48*, 497−507.

(3) Mortelmans, K.; Zeiger, E. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **2000**, *455*, 29−60.

(4) Schmid, W. *Mutat. Res.* **1975**, *31*, 9−15.

(5) Glowienke, S.; Frieauff, W.; Allmendinger, T.; Martus, H. J.; Suter, W.; Mueller, L. *Mutat. Res.* **2005**, *581*, 23−34.

(6) Raman, N. V. V. S. S.; Prasad, A. V. S. S.; Reddy, K. R. *J. Pharmaceut. Biomed. Anal.* **2011**, *55*, 662−667.

(7) *Impurities in New Drug Substances*, Guideline Q3A(R2); International Conference on Harmonisation (ICH): Geneva, Switzerland, 2006.

(8) *Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk*, Guideline M7; International Conference on Harmonisation (ICH): Geneva, Switzerland, 2013.

(9) Giordani, A.; Kobel, W.; Gally, H. U. *Eur. J. Pharm. Sci.* **2011**, *43*, 1−15.

(10) Li, Y.; Liu, D. Q.; Yang; Sudini, S. R.; McGuire, M. A.; Bhanushali, D. S.; Kord, A. S. *J. Pharmaceut. Biomed. Anal.* **2010**, *52*, 493−507.

(11) Szekely, G.; Bandarra, J.; Heggie, W.; Sellergren, B.; Ferreira, F. C. *J. Membr. Sci.* **2011**, *381*, 21−33.

(12) Elder, D. P.; Delaney, E.; Teasdale, A.; Eyley, S.; Reif, V. D.; Jacq, K.; Facchine, K. L; Schulte Oestrich, R.; Sandra, P.; David, F. *J. Pharm. Sci.* **2010**, *99*, 2948−2961.

(13) Liu, D. Q.; Sun, M.; Kord, A. S. *J. Pharmaceut. Biomed. Anal.* **2010**, *51*, 999−1014.

(14) Ramakrishna, K.; Raman, N. V. V. S. S.; Rao, K. M. V.; Prasad, A. V. S. S.; Reddy, K. S. *J. Pharmaceut. Biomed. Anal.* **2008**, *46*, 780−783.

(15) Wollein, U.; Schramek, N. *Eur. J. Pharm. Sci.* **2012**, *45*, 201−204.

(16) Li, W. Y. *J. Chromatogr. A* **2004**, *1046*, 297−301.

(17) Alzaga, R.; Ryan, R. W.; Taylor-Worth, K. A. M.; Lipczynski, K. A. M.; Szucs, R.; Sandra, P. *J. Pharmaceut. Biomed. Anal.* **2007**, *45*, 472−479.

(18) Jacq, K.; Delaney, E.; Teasdale, A.; Eyley, S.; Taylor-Worth, K.; Lipczynski, A.; Reif, V. D.; Elder, D. P.; Facchine, K. L.; Golec, S.; Oestrich, R. S.; Sandra, P.; David, F. *J. Pharmaceut. Biomed. Anal.* **2008**, *48*, 1339−1344.

(19) Colon, I.; Richoll, S. M. *J. Pharmaceut. Biomed. Anal.* **2005**, *39*, 477−485.

(20) An, J.; Sun, M.; Bai, L.; Chen, T.; Liu, D. Q.; Kord, A. *J. Pharmaceut. Biomed. Anal.* **2008**, *48*, 1006−1010.

(21) Taylor, G. E.; Gosling, M.; Pearce, A. *J. Chromatogr. A* **2006**, *1119*, 231−237.

(22) Kakadiya, P. R.; Reddy, B. P.; Singh, V.; Ganguly, S.; Chandrashekhar, T. G.; Singh, D. K. *J. Pharmaceut. Biomed. Anal.* **2011**, *55*, 379−384.

(23) McCullough, B. J.; Bristow, T.; O'Connor, G.; Hopley, C. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 1445−1451.

(24) Chen, H.; Zenobi, R. *Nat. Protoc.* **2008**, *3*, 1467−1475.

(25) U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM). *Guidance for Industry: Bioanalytical Method Validation*; Technical Report; Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM): Rockville, MD, May 2001; 25 pp.